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14. ABSTRACT

The PCRP Prostate Cancer Training Award has supported my training for the past 12 months. During this time, I have continued to work towards attaining a better understanding of (a) the biology of prostate cancer, (b) the biology of the Wnt/beta-catenin signaling pathway, (c) the principle and application of cutting edge techniques in modern molecular and cellular biology, and (d) the principle and application of genetically engineered mouse (GEM) models, with the intent of becoming uniquely positioned to investigate the role of Wnt/beta-catenin signaling in the initiation, progression and metastasis of spontaneous autochthonous prostate cancer and the emergence of the androgen depletion independent phenotype. In addition to this rigorous technical training, I have continued to attend weekly lab meetings, division wide Cancer Biology Seminar series, and the Pacific Northwest SPORE in Prostate Cancer meetings. In addition, I attended the PCRP's Innovative Minds in Prostate Cancer Today (IMPaCT) meeting in Atlanta, Georgia.

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Progress Report
PCRP Prostate Cancer Training Award #W81XWH-07-1-0039
PI: Scott Houghtaling, Ph.D.

Introduction:
The PCRP Prostate Cancer Training Award has supported my training for the past 9 months. During this time, I have continued to work towards attaining a better understanding of (a) the biology of prostate cancer, (b) the biology of the Wnt/beta-catenin signaling pathway, (c) the principle and application of cutting edge techniques in modern molecular and cellular biology, and (d) the principle and application of genetically engineered mouse (GEM) models, with the intent of becoming uniquely positioned to investigate the role of Wnt/beta-catenin signaling in the initiation, progression and metastasis of spontaneous autochthonous prostate cancer and the emergence of the androgen depletion independent phenotype. In addition to this rigorous technical training, I have continued to attend weekly lab meetings, division wide Cancer Biology Seminar series, and the Pacific Northwest SPORE in Prostate Cancer meetings. In addition, I attended the PCRP's Innovative Minds in Prostate Cancer Today (IMPaCT) meeting in Atlanta, Georgia.

Body:
Specific aim 1: Determine the influence of enforced expression of the proto-oncogene, *Wnt3*, on epithelial cells of the mouse prostate.
We have engineered the bi-cistronic plasmid, *PB/Wnt3-IRES-Luc* (Figure 1A). We have tested the Wnt3 coding sequence in cell culture experiments to show that expression of *Wnt3* is capable of activating the canonical Wnt signaling pathway (Figure 1B). Briefly, 293HEK cells were seeded in triplicate and transfected with *pcDNA3.1/Wnt3*, pRenilla, and pSuperTopFlash (Wnt signaling reporter plasmid kindly provided by the R. Moon lab, Seattle, WA). Cell lysates were prepared 24 hours after transfection and relative levels of Firefly Luciferase were measured as an indication of canonical Wnt signaling activity. We have also tested the androgen responsiveness of *PB/Wnt3-IRES-Luc* by measuring Luciferase expression in PC3 prostate cancer cells co-transfected with *PB/Wnt3-IRES-Luc* and *pcDNA3.1/Androgen Receptor* (AR) grown in the presence or absence of synthetic androgen (R1881) (Figure 1C). In preparation for microinjection into FVB zygotes, *PB/Wnt3-IRES-Luc* was digested with NotI/XhoI and a 4829 bp fragment containing the transgene was gel purified. We have generated 2 independent lines and analyzed them for reporter gene expression (Luciferase) using the Xenogen IVIS system (Figure 2). While the expression of Luciferase was observed in the prostate of these animals, it was not spatially restricted to this organ.

To provide better spatially restricted expression from the Probasin promoter, we reengineered our transgene construct to include 2 tandem repeats of the chicken β-globin HS4 region that functions as an insulator. This modified transgene was used for transgenesis in FVB zygotes. We observed a nearly identical pattern of Luciferase reporter expression in multiple founders with the modified transgene (data not shown).

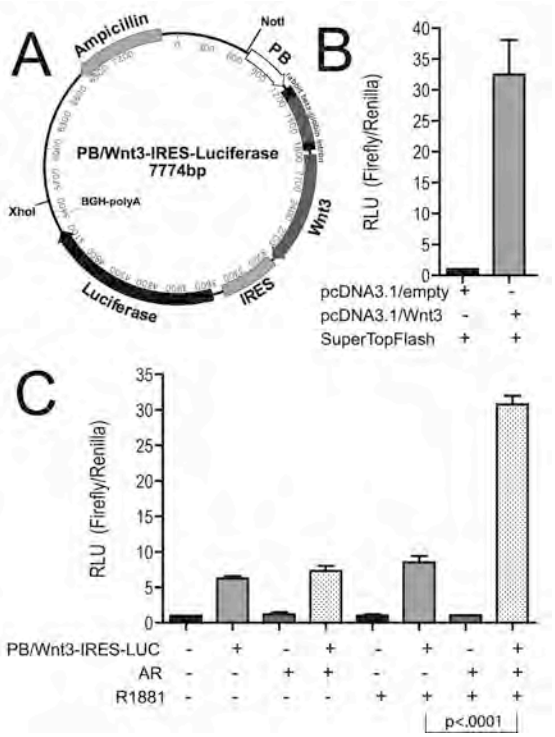

Figure 1. Analysis of transgene construct. A. Transgene construct showing probasin (PB) promoter driving *Wnt3* transgene expression. B. Luciferase assay showing active Wnt signaling in 293HEK cells transfected with SuperTopFlash reporter plasmid and *Wnt3* coding sequence or empty vector control. C. Lucfierase assay showing prostate cell specific transgene expression in PC3 prostate cancer cells. Columns represent mean +/- SEM.

We believe that inappropriate expression of *Wnt3* during development may negatively select against embryos with high level of transgene expression. This is consistent with the observation of transgenic founder animals with widespread, low level expression of the luciferase reporter. To develop mice with high level, prostate specific expression of Wnt3/Luciferase, we have decided to engineer a knockin model. The theoretical construct is show in Figure 3. Three copies of a strong transcriptional stop signal, flanked by lox p sites, will be inserted between the constitutively active CAG promoter (a combination of the chicken beta-actin promoter and cytomegalovirus immediate-early enhancer) and the downstream Wnt3/Luciferase bicistronic transgene. The entire transgene will be "knocked-in" to the ROSA locus via homologous recombination in mouse ES cells. The resulting "knock-in" mice will be crossed to ARR_2PB-Cre mice in which expression of Cre recombinase is under control of the modified Probasin promoter. Bigenic (CAG/Wnt3-Luciferase; ARR_2PB/Cre) mice will have the transcriptional stop signal deleted in the prostate epithelium and Wnt3/Luiferase expression should be restricted to epithelial cells of the mouse prostate.

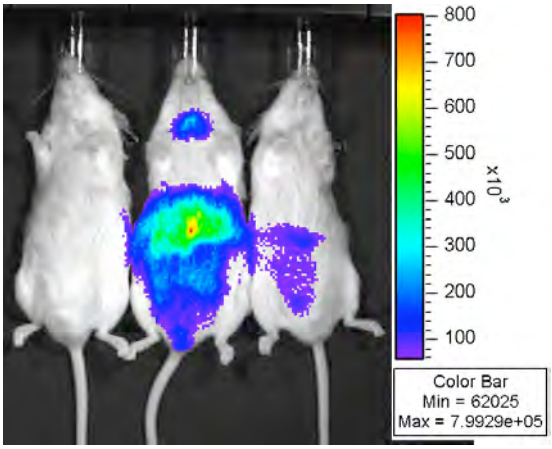

Figure 2. Imaging of PB/Wnt3-IRES-Luciferase mice. Mice were anesthetized with a mixture of 1.5% isoflurane/air. D-luciferin was i.p. injected at 25 mg/kg mouse body weight. Ten minutes after injection, mice were imaged with an IVIS Imaging System™ (Xenogen) with continuous isoflurane exposure. [L to R] Neg. control, founder, Pos. control (EZC).

Specific aim 2: Determine the influence of enforced Wnt3 expression during tumor progression and metastasis in the transgenic adenocarcinoma of the mouse prostate (TRAMP) model.
We are waiting to select the CAG/loxSTOPlox/*Wnt3*-IRES-*Luc* transgenic line with the highest spatially restricted expression of *Wnt3* prior to crossing to TRAMP.

Specific aim 3: Develop lines of reporter mice to detect activation of the Wnt/β-catenin pathway *in vivo* during normal development and cancer progression.
We obtained the plasmid, 16xSuperTopFlash (16xSTF), from the Moon lab (University of Washington) and re-engineered the plasmid to contain a loxp-STOPx3-loxp cassette positioned upstream of the Luciferase ORF (Figure 4). Tissue specificity for the reporter transgene will be obtained by crossing the Wnt reporter mice to transgenic mice with Cre recombinase driven by tissue specific promoter, (eg. ARR_2PB/Cre). We plan to test the reporter construct in a cell culture system by transfecting 293HEK cells with our modified reporter construct, pcDNA3.1/Cre, and pcDNA3.1/Wnt3 followed by a Luciferase assay to measure activation of the reporter. The plasmid will be digested with KpnI/SalI, a 4345 bp fragment will be gel purified and prepared for microinjection.

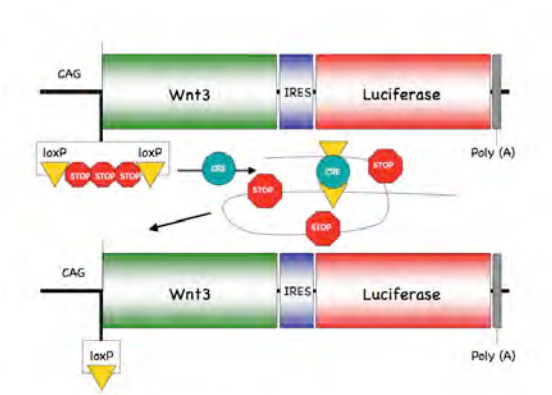

Figure 3. Knock-in construct showing three copies of a strong transcriptional stop signal, flanked by lox p sites, will be inserted between the constitutively active CAG promoter (a combination of the chicken beta-actin promoter and cytomegalovirus immediate-early enhancer) and the downstream Wnt3/Luciferase bicistronic transgene. Flanking arms, used for homologous recombination into ROSA locus, are not shown.

Key Research Accomplishments: none

Reportable Outcomes: none

Conclusions:

The results of this work should support or refute the hypothesis that enforced expression of *Wnt3* is sufficient to drive tumorigenesis in the mouse prostate and may provide support for Wnt3 in metastatic disease. A quantitative increase in the GU weight of bigenic (CAG/loxSTOPlox/*Wnt3*-IRES-*Luc* x TRAMP) animals compared to TRAMP mice would support the central hypothesis that deregulated Wnt signaling can promote prostate cancer in the mouse.

Furthermore, an increase in the frequency or size of osteoblastic bone metastases in bigenic mice compared to age matched TRAMP mice would support the hypothesis that activated Wnt signaling in tumor cells can promote the formation of bone metastases.

We continue to generate CAG/loxSTOPlox/*Wnt3*-IRES-*Luc* knock-in mice. Founder animals will be imaged at 12, 18, and 24 weeks using a Xenogen IVIS to detect Luciferase expression. Prostates from transgenic mice will be analyzed for cell proliferation, apoptosis, changes in morphology and pathology. The best expressing lines will be crossed to TRAMP mice.

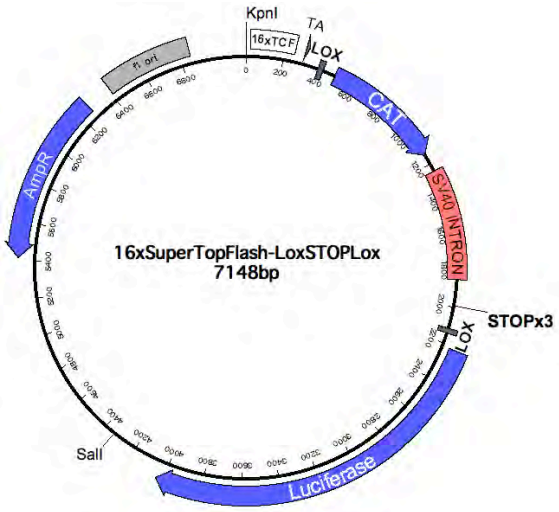

Figure 4. The Wnt Reporter plasmid has been modified to contain a Lox-STOPx3-Lox cassette to provide tissue specificity.

References: none

Appendices: none